UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLUTTION PREVENTION

MEMORANDUM

DATE: October 13, 2020

SUBJECT: Efficacy Review for T-BONE
EPA Reg. No. 777-139
DP Barcode: 458561
E-Submission: 52093

From: Tahirah Burford
Efficacy Branch
Antimicrobials Division (7510P)
Date Signed: November 20, 2020

Thru: Thao Pham
Efficacy Branch
Antimicrobials Division (7510P)
Date Signed: November 20, 2020

To: Lorena Rivas / Jacqueline Hardy
Regulatory Management Branch II
Antimicrobials Division (7510P)

APPLICANT: Reckitt Benckiser
Morris Corporate Center IV
399 Interpace Parkway
Parsippany, NJ 07054-0025

FORMULATION FROM LABEL:

Active Ingredient	% by wt.
Citric acid	2.78%
Other ingredients	97.22%
Total	100.00%

I. BACKGROUND

Product Description (as packaged, as applied): Ready-to-use Pre-saturated towelette

Submission type: Label Amendment

Currently registered efficacy claim(s): One-step disinfectant (bactericide, virucide, fungicide), non-food contact sanitizer, and deodorizer for use on hard, non-porous surfaces.

Requested action(s): Add disinfection and soft surface sanitization claims against various microorganisms.

Documents considered in this review:

- A Cover letter to EPA dated 07/01/2020
- Confidential Statement of Formula (EPA form 8570-4) dated 07/01/2020
- Data Matrix (EPA form 8570-35) dated 07/01/2020
- 24 efficacy studies (MRID No. 51196005-51196028)
- Proposed product label dated 07/01/2020

II. USE DIRECTIONS

To Sanitize / Disinfect (on hard non-porous surfaces) (and / &) (semi critical medical device or medical equipment surfaces): (Pre-clean surface.) Use a fresh wipe to thoroughly wet surface. (Surface must remain wet for the entire contact time.) Leave for 10 seconds to sanitize. Leave for 4 minutes to disinfect. Air dry. (Rinse all food contact surfaces and toys with water after use.) (Discard in trash.)

(To Spot Sanitize (Soft Surfaces) (Fabrics): Wipe the surface (&) (ensure it remains) (leave) wet for at least 4 minutes. Let air dry. (For difficult odors or heavy fabrics, repeat application).)

(**For surfaces that come in contact with food**: Use only on hard, non-porous surfaces and rinse thoroughly with water.)

(To Disinfect Toys: Use only on hard, non-porous surfaces and rinse thoroughly with water after use.)

<< USE DIRECTIONS - FOR CONVENIENCE MOP >>
To Sanitize / Disinfect (on hard non-porous surfaces) (and / &) (semi critical medical device or medical equipment surfaces): (Pre-clean surface.) Use a fresh wipe to thoroughly wet surface. (Surface must remain wet for the entire contact time.) Leave for 10 seconds to sanitize. Leave for 4 minutes to disinfect. Air dry. (Discard in trash.)

III. STUDY SUMMARIES

1. **MRID 511960-05, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: *Bordetella pertussis* (ATCC 12743) for product T-Bone US (777-139), Lots # 2290-022 and # 2290-023. Study conducted at Accuratus Lab Services by Kristin Hunt, B.S. Study completion date – September 11, 2019. Project Number A27910.**

This study was conducted against *Bordetella pertussis* (ATCC 12743). Two lots (Lot Nos. 2290-022 and 2290-023) of the product, T-Bone US at LCL Formula e0143-166B (777-139), were tested using Accuratus Protocol No. REK01041019.TOW.2. The product was received ready-to-use as a pre-saturated towelette. From stock, sufficient agar plates were inoculated with the test organism and incubated for 3-5 days (4 days) at 35-37°C (36.0°C). Following incubation, the culture was suspended in Butterfield's buffer to approximately target a 3.0 McFarland turbidity standard. The culture was diluted using sterile growth medium by combining 1.00 mL of test organism suspension with 49.0 mL of Butterfield's Buffer. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri

dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 35-37°C (35.9-36.0°C) and 47.6-53.3% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (20°C) and 50% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. The subcultures were vortex mixed for 120±5 seconds. Within 30 minutes of vortex mixing, the entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.2 µm porosity) pre-wetted with 10 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline, removed aseptically from the filter unit and placed on the surface of a Bordet Gengou Agar plate for recovery of the test organism. All subcultures were incubated for 5 days at 35-37°C (36.0°C). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: No protocol deviations were required; protocol amendments in this study were reviewed and found to be acceptable.

2. **MRID 511960-06, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: *Vibrio cholerae* (ATCC 14035) for product T-Bone US (777-139), Lots # 2290-022 and # 2290-023. Study conducted at Accuratus Lab Services by Kristin Hunt, B.S. Study completion date – September 10, 2019. Project Number A27914.**

This study was conducted against *Vibrio cholerae* (ATCC 14035). Two lots (Lot Nos. 2290-022 and 2290-023) of the product, T-Bone US at LCL Formula e0143-166B (777-139), were tested using Accuratus Protocol No. REK01041019.TOW.11. The product was received ready-to-use as a pre-saturated towelette. From stock, sufficient agar plates were inoculated with the test organism and incubated for 2 days at 35-37°C (36.0°C). The culture was adjusted using Fluid Thioglycolate Medium to target a spectrophotometer absorbance reading of ~2.3 (2.323) at 620 nm. The culture was diluted using Fluid Thioglycolate Medium by combining 1.00 mL of test organism suspension with 14.0 mL of diluent. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 27 minutes at 25-30°C (26.8-27.7°C) and 62-64% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (19°C) and 54% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 2-3 days (2 days) at 35-37°C (36.0°C). Subcultures were stored at 2-8°C for one day prior to examination.

Following incubation and storage, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: No protocol deviations were required; protocol amendments in this study were reviewed and found to be acceptable.

3. **MRID 511960-07, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: *Streptococcus mutans* (ATCC 25175) for product T-Bone (777-139), Lots # 2290-022 and # 2290-023. Study conducted at Accuratus Lab Services by James Walrath, B.S. Study completion date – September 6, 2019. Project Number A28016.**

This study was conducted against *Streptococcus mutans* (ATCC 25175). Two lots (Lot Nos. 2290-022 and 2290-023) of the product, T-Bone US at LCL Formula e0143-166B (777-139), were tested using Accuratus Protocol No. REK01041019.TOW.6. The product was received ready-to-use as a pre-saturated towelette. A loopful of stock slant culture was transferred to an initial 10 mL tube of Brain Heart Infusion Broth growth medium. The tube was mixed and incubated for 24±2 hours at 35-37ºC (36ºC). Following incubation, a 10µL aliquot of culture was transferred to sufficient 20 x 150 mm Morton Closure tubes containing 10 mL of culture medium (1st daily transfer). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours (48 hours) at 35-37ºC (36ºC). The test culture was vortex mixed (3-4 seconds) and allowed to stand for ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 1.00 mL of test organism suspension with 14.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 36 minutes at 25-30°C (26.6-27.0°C) and 63-67% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (21°C) and 53% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Brain Heart Infusion Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 2-4 days (2 days) at 35-37°C (36.0°C) in 6.0% CO_2. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: No protocol amendments were required; protocol deviations in this study were reviewed and found to be acceptable.

Note: Testing performed on July 5, 2019 resulted in the carrier population control exceeding an average Log10 value of 5.0 and the test substance did not meet the performance criteria. Testing was repeated on July 23, 2019.

4. **MRID 511960-08, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: *Shigella dysenteriae* (ATCC 11835) for product T-Bone US (777-139), Lots # 2290-022 and # 2290-023. Study conducted at Accuratus Lab Services by Kristin Hunt, B.S. Study completion date – September 10, 2019. Project Number A27913.**

This study was conducted against *Shigella dysenteriae* (ATCC 11835). Two lots (Lot Nos. 2290-022 and 2290-023) of the product, T-Bone US at LCL Formula e0143-166B (777-139), were tested using Accuratus Protocol No. REK01041019.TOW.10. The product was received ready-to-use as a pre-saturated towelette. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium. The tube was mixed and incubated for 24±2 hours at 35-37ºC (36ºC). Following incubation, a 10µL aliquot of culture was transferred to sufficient 20 x 150 mm Morton Closure tubes containing 10 mL of culture medium (1st daily transfer). Four additional daily transfers were prepared. The final test culture was incubated for 48-54 hours (48 hours) at 35-37ºC (36ºC). The test culture was vortex mixed (3-4 seconds) and allowed to stand for ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was diluted by combining 1.0 mL of test organism suspension with 4.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 25-30°C (26.7-26.8°C) and 63-64% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (19°C) and 53% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (47.25 hours) at 35-37°C (36.0°C). Subcultures were stored at 2-8°C for one day prior to examination. Following incubation and storage, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: Protocol amendments and deviations in this study were reviewed and found to be acceptable.

5. **MRID 511960-09, "A GLP Evaluation of a Disinfectant Towelette". Test Organism: Vancomycin-Resistant *Enterococcus faecium* (ATCC BAA-2320) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Bioscience Laboratories, Inc. by Terri Eastman. Study completion date – April 7, 2020. Laboratory Study Number 2001488-204B.**

This study was conducted against Vancomycin-Resistant *Enterococcus faecium* (ATCC BAA-2320). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Bioscience Laboratories Study No. 2001488-204B. The product was received ready-to-use as a pre-saturated towelette. A 10µL aliquot of thawed

stock culture was transferred to a tube containing 10 mL of Nutrient Broth. The tube was mixed and incubated at 36±1ºC (35.7-36.2ºC test date 2/20/20; 35.8-35.9 ºC test day 3/10/20) for 24±2 hours (22 hours 35 minutes test date 2/20/20; 22 hours 29 minutes test date 3/10/20). The 24-hr broth culture was briefly vortexed, and 10µL aliquots were transferred to a number of tubes containing 10 mL of Nutrient Broth to ensure an inoculum volume sufficient for testing. The tubes were incubated for 48-54 hours (48 hours test date 2/20/20; 48 hours 1 minute test date 3/10/20) at 36±1ºC (36.2-36.3ºC test date 2/20/20; 35.9-36.2ºC test date 3/10/20). The test culture was vortex mixed (3-4 seconds) and allowed to stand for 10 minutes at room temperature. The upper portion was removed and pooled separately in a sterile container. Heat inactivated fetal bovine serum was added to the inoculum suspension to achieve a 5% organic soil load. The final test culture was mixed thoroughly prior to use. Individual glass slide carriers were inoculated with 0.01 mL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 36.5-36.6°C and 20.61-22.12% relative humidity (test date 2/20/20) and 36.1-36.3°C and 16.14-20.40% relative humidity (test date 3/10/20). The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium Letheen Broth (0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (48 hours 11 minutes test date 2/20/20; 46 hours 2 minutes test date 3/10/20) at 36±1°C (36.5-36.6°C test date 2/20/20; 36.3-36.4°C test date 3/10/20). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier counts.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments or deviations were reported in this study.

6. MRID 511960-10, "A GLP Evaluation of a Disinfectant Towelette". Test Organism: 3rd Generation Cephalosporin-Resistant *Escherichia coli* (ATCC BAA-2780) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Bioscience Laboratories, Inc. by Terri Eastman. Study completion date – April 7, 2020. Laboratory Study Number 2001488-204C.

This study was conducted against 3rd Generation Cephalosporin-Resistant *Escherichia coli* (ATCC BAA-2780). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Bioscience Laboratories Study No. 2001488-204C. The product was received ready-to-use as a pre-saturated towelette. A 10µL aliquot of thawed stock culture was transferred to a tube containing 10 mL of Nutrient Broth. The tube was mixed and incubated at 36±1ºC (36.0-36.1ºC) for 24±2 hours (23 hours 44 minutes). The 24-hr broth culture was briefly vortexed, and 10µL aliquots were transferred to a number of tubes containing 10 mL of Nutrient Broth to ensure an inoculum volume sufficient for testing. The tubes were incubated for 48-54 hours (47 hours 56 minutes) at 36±1ºC (36.1-36.3ºC). The test culture was vortex mixed (3-4 seconds) and allowed to stand for 10 minutes at room temperature. The upper portion was removed and pooled separately in a sterile container. Heat inactivated fetal bovine serum was added to the inoculum suspension to achieve a 5%

organic soil load. The final test culture was mixed thoroughly prior to use. Individual glass slide carriers were inoculated with 0.01 mL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 21 minutes at 36±1ºC (36.2-36.4ºC) and 20.00-24.03% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium Letheen Broth (0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (46 hours 22 minutes) at 36±1°C (35.6-36.0°C). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier counts.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments were required; protocol deviations in this study were reviewed and found to be acceptable.

7. **MRID 511960-11, "A GLP Evaluation of a Disinfectant Towelette". Test Organism: 3rd Generation Cephalosporin-Resistant *Klebsiella pneumoniae* (ATCC BAA-2784) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Bioscience Laboratories, Inc. by Terri Eastman. Study completion date – April 21, 2020. Laboratory Study Number 2001488-204D.**

This study was conducted against 3rd Generation Cephalosporin-Resistant *Klebsiella pneumoniae* (ATCC BAA-2784). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Bioscience Laboratories Study No. 2001488-204D. The product was received ready-to-use as a pre-saturated towelette. A 10µL aliquot of thawed stock culture was transferred to a tube containing 10 mL of Nutrient Broth. The tube was mixed and incubated at 36±1ºC (36.0-36.1ºC test date 2/19/20; 35.8-35.9 ºC test day 3/10/20) for 24±2 hours (23 hours 44 minutes test date 2/19/20; 22 hours 29 minutes test date 3/10/20). The 24-hr broth culture was briefly vortexed, and 10 µL aliquots were transferred to a number of tubes containing 10 mL of Nutrient Broth to ensure an inoculum volume sufficient for testing. The tubes were incubated for 48-54 hours (47 hours 56 minutes test date 2/19/20; 48 hours 1 minute test date 3/10/20) at 36±1ºC (36.1-36.3ºC test date 2/19/20; 35.9-36.2ºC test date 3/10/20). The test culture was vortex mixed (3-4 seconds) and allowed to stand for 10 minutes at room temperature. The upper portion was removed and pooled separately in a sterile container. Heat inactivated fetal bovine serum was added to the inoculum suspension to achieve a 5% organic soil load. The final test culture was mixed thoroughly prior to use. Individual glass slide carriers were inoculated with 0.01 mL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 36.3-36.4°C and 21.69-23.10% relative humidity (test date 2/19/20) and 35.9-36.3°C and 14.65-29.65% relative humidity (test date 3/10/20). The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and

each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium Letheen Broth (0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (46 hours 6 minutes test date 2/19/20; 46 hours 2 minutes test date 3/10/20) at 36±1°C (35.7-35.9°C test date 2/19/20; 36.3-36.4°C test date 3/10/20). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier counts.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments were required; protocol deviations in this study were reviewed and found to be acceptable.

8. **MRID 511960-12, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-021. Study conducted at Analytical Lab Group-Midwest by Thomas Breyen, B.S. Study completion date – April 9, 2020. Project Number A29137.**

This study was conducted against Vancomycin Intermediate Resistant *Staphylococcus aureus* - VISA (CDC HIP 5836). Two lots (Lot Nos. 2315-010 and 2315-021) of the product, T-Bone US e0143-166B (777-139), were tested using Analytical Lab Group-Midwest Protocol No. REK01011720.TOW.1. The product was received ready-to-use as a pre-saturated towelette. From stock, sufficient agar plates were inoculated with the test organism and incubated for 2 days at 35-37°C (36.0°C). Following incubation, the culture was suspended in sterile Butterfield's buffer and adjusted to target a spectrophotometer absorbance reading between 0.240 and 0.260 (0.260) at 620 nm. The culture was then diluted using sterile growth medium by combining 0.20 mL of test organism suspension with 5.8 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 35-37°C (35.9-36.3°C) and 50.7-59.4% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (20°C) and 17% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (47.25 hours) at 35-37°C (36.0°C). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day

of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol deviations were required; protocol amendments in this study were reviewed and found to be acceptable.

9. **MRID 511960-13, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: Carbapenem Resistant *Escherichia coli* (CDC 81371) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Analytical Lab Group-Midwest by Jamie Herzan, B.S. Study completion date – April 15, 2020. Project Number A29148.**

This study was conducted against Carbapenem Resistant *Escherichia coli* (CDC 81371). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Analytical Lab Group-Midwest Protocol No. REK01011720.TOW.2. The product was received ready-to-use as a pre-saturated towelette. A loopful of stock slant culture was transferred to an initial 10 mL tube of Synthetic Broth growth medium. The tube was mixed and incubated for 24±2 hours at 35-37ºC (36ºC). Following incubation, a 10µL aliquot of culture was transferred to sufficient 20 x 150 mm Morton Closure tubes containing 10 mL of culture medium (1st daily transfer). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours (48 hours) at 35-37ºC (36ºC). The test culture was vortex mixed (3-4 seconds) and allowed to stand for ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was adjusted using sterile growth medium to target a spectrophotometer absorbance reading between 0.240 and 0.260 (0.247) at 620 nm. The culture was diluted by combining 1.00 mL of test organism suspension with 4.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 35-37°C (36.2-36.6°C) and 41.6-50.4% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (20°C) and 12% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (46.25) at 35-37°C (36.0°C). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments or deviations were reported in this study.

10. **MRID 511960-14, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study**

conducted at Analytical Lab Group-Midwest by Jamie Herzan, B.S. Study completion date – April 15, 2020. Project Number A29162.

This study was conducted against Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Analytical Lab Group-Midwest Protocol No. REK01011720.TOW.4. The product was received ready-to-use as a pre-saturated towelette. From stock, sufficient agar plates were inoculated with the test organism and incubated for 2 days at 35-37°C (36.0°C). Following incubation, the culture was suspended in sterile Butterfield's buffer to target a spectrophotometer absorbance reading between 0.240 and 0.260 (0.247) at 620 nm. The culture was then diluted by combining 1.00 mL of test organism suspension with 24.0 mL of sterile Butterfield's Buffer. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 35-37°C (36.0-36.2°C) and 51.8-52.6% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (20°C) and 12% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (48 hours) at 35-37°C (36.0°C). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments or deviations were reported in this study.

11. **MRID 511960-15, "A GLP Evaluation of a Disinfectant Towelette". Test Organism: Multidrug-Resistant *Klebsiella aerogenes* (AR-Bank 0547) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Bioscience Laboratories, Inc. by Terri Eastman. Study completion date – April 21, 2020. Laboratory Study Number 2001488-204F.**

This study was conducted against Multidrug-Resistant *Klebsiella aerogenes* (AR-Bank 0547). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Bioscience Laboratories Study No. 2001488-204F. The product was received ready-to-use as a pre-saturated towelette. A 10µL aliquot of thawed stock culture was transferred to a tube containing 10 mL of Nutrient Broth. The tube was mixed and incubated at 36±1ºC (36.1-36.2ºC) for 24±2 hours (23 hours 3 minutes). The 24-hr broth culture was briefly vortexed, and 10µL aliquots were transferred to a number of tubes containing 10 mL of Nutrient Broth to ensure an inoculum volume sufficient for testing. The tubes were incubated for 48-54 hours (48 hours 1 minute) at 36±1ºC (36.1-36.2ºC). The test culture was vortex mixed (3-4 seconds) and allowed to stand for 10 minutes at room temperature. The upper portion was removed and pooled separately in a sterile container.

Heat inactivated fetal bovine serum was added to the inoculum suspension to achieve a 5% organic soil load. The final test culture was mixed thoroughly prior to use. Individual glass slide carriers were inoculated with 0.01 mL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 36±1ºC (36.2ºC) and 22.95-25.31% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium Letheen Broth (0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (46 hours 50 minutes) at 36±1°C (35.7-36.0°C). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier counts.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: Protocol amendments and deviations in this study were reviewed and found to be acceptable.

12. **MRID 511960-16, "A GLP Evaluation of a Disinfectant Towelette". Test Organism: Carbapenem-Resistant *Acinetobacter baumannii* (ATCC BAA-2901) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Bioscience Laboratories, Inc. by Terri Eastman. Study completion date – April 21, 2020. Laboratory Study Number 2001488-204A.**

This study was conducted against Carbapenem-Resistant *Acinetobacter baumannii* (ATCC BAA-2901). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Bioscience Laboratories Study No. 2001488-204A. The product was received ready-to-use as a pre-saturated towelette. A 10µL aliquot of thawed stock culture was transferred to a tube containing 10 mL of Nutrient Broth. The tube was mixed and incubated at 36±1ºC (36.0-36.1ºC test date 2/18/20; 35.8-35.9 ºC test day 3/10/20) for 24±2 hours (22 hours 40 minutes test date 2/18/20; 22 hours 29 minutes test date 3/10/20). The 24-hr broth culture was briefly vortexed, and 10 µL aliquots were transferred to a number of tubes containing 10 mL of Nutrient Broth to ensure an inoculum volume sufficient for testing. The tubes were incubated for 48-54 hours (48 hours 3 minutes test date 2/18/20; 48 hours 1 minute test date 3/10/20) at 36±1ºC (36.1ºC test date 2/18/20; 35.9-36.2ºC test date 3/10/20). The test culture was vortex mixed (3-4 seconds) and allowed to stand for 10 minutes at room temperature. The upper portion was removed and pooled separately in a sterile container. Heat inactivated fetal bovine serum was added to the inoculum suspension to achieve a 5% organic soil load. The final test culture was mixed thoroughly prior to use. Individual glass slide carriers were inoculated with 0.01 mL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 35.2-36.0°C and 10.88-17.16% relative humidity (test date 2/18/20) and 35.8-35.9°C and 14.70-17.52% relative humidity (test date 3/10/20). The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals.

The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium Letheen Broth (0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (46 hours test date 2/18/20; 46 hours 2 minutes test date 3/10/20) at 36±1°C (36.2-36.6°C test date 2/18/20; 36.3-36.4°C test date 3/10/20). Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier counts.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments or deviations were reported in this study.

13. MRID 511960-17, "Pre-Saturated Towelettes for Hard Surface Disinfection". Test Organism: Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) for product T-Bone US (777-139), Lots # 2315-010 and # 2315-011. Study conducted at Analytical Lab Group-Midwest by Thomas Breyen, B.S. Study completion date – April 10, 2020. Project Number A29138.

This study was conducted against Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705). Two lots (Lot Nos. 2315-010 and 2315-011) of the product, T-Bone US e0143-166B (777-139), were tested using Analytical Lab Group-Midwest Protocol No. REK01011720.TOW.3. The product was received ready-to-use as a pre-saturated towelette. A loopful of stock slant culture was transferred to an initial 10 mL tube of Nutrient Broth growth medium. The tube was mixed and incubated for 24±2 hours at 35-37ºC (36ºC). Following incubation, a 10µL aliquot of culture was transferred to sufficient 20 x 150 mm Morton Closure tubes containing 10 mL of culture medium (1st daily transfer). One additional daily transfer was prepared. The final test culture was incubated for 48-54 hours (48 hours) at 35-37ºC (36ºC). The test culture was vortex mixed (3-4 seconds) and allowed to stand for ≥10 minutes prior to use. The upper portion was removed and pooled in a sterile vessel and mixed. The culture was adjusted using sterile growth medium to target a spectrophotometer absorbance reading between 0.240 and 0.260 (0.254) at 620 nm. The culture was diluted by combining 1.00 mL of test organism suspension with 19.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Individual glass slide carriers were inoculated with 10.0 µL of culture using a calibrated pipettor spreading the inoculum uniformly over an ~1 inch x 1 inch area on the end of the slide contained in the Petri dish and covered immediately. The culture was vortex mixed periodically during inoculation as necessary. The slides were allowed to dry for 30 minutes at 35-37°C (36.1°C) and 49.0-49.2% relative humidity. The carriers were used in the test procedure within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (20°C) and 12% relative humidity for 3 minutes 50 seconds in a horizontal and undisturbed fashion. Following exposure, excess liquid was drained off and each carrier was transferred using sterile forceps at staggered intervals to 20 mL of neutralizing subculture medium (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) and each vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (47 hours) at 35-37°C (36.0°C). Following incubation, the subcultures were visually examined for the

presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation and carrier population.

Note: Antibiotic sensitivity testing was performed using a representative culture from the day of testing and verified the antibiotic resistance pattern of the test organism.

Note: No protocol amendments or deviations were reported in this study.

14. MRID 511960-18, "Viral Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection." Virus: Herpes simplex virus type 1 for product T-Bone (Batch 2290-22 and Batch 2295-009). Study conducted at Accuratus Lab Services by Matt Cantin, B.S. Study completion date – September 11, 2019. Project Number A27988.

This study was conducted against the F(1) strain of Herpes simplex virus type 1 (ATCC VR-733) for product T-Bone Formula #e0143-166B (Batch 2290-22 and Batch 2295-009) using Accuratus Protocol No. REK01041019.HSV1 (copy provided). Stock virus obtained from ATCC was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approx. 2000 RPM for 5 minutes at approx. 4°C. The supernatant was removed, aliquoted and the high titer stock virus was stored at ≤-70°C. On the day of use, an aliquot of stock virus (Accuratus Lot H87) was thawed, maintained at a refrigerated temperature until used in the assay, and contained 5% fetal bovine serum (FBS) as the organic soil load. The culture demonstrated cytopathic effects (CPE) typical of Herpes simplex virus on Vero cells. Indicator Vero cells originally obtained from ATCC (ATCC CCL-81) were propagated at Accuratus Lab Services, seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. On the day of testing, the cells were observed as having proper cell integrity and confluency. Test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat inactivated FBS, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Single use towelettes impregnated with the active ingredient were supplied by the Sponsor and used per the Sponsor's direction. The towelettes were folded widthwise two times and lengthwise two times to form an approximate 2 x 2 inch square. These were pre-equilibrated to exposure temperature prior to use. Films of dried virus were prepared by spreading 200 µL of virus inoculum uniformly over a defined area (approx. 8 x 8 cm) on the bottoms of 3 separate carriers (150 x 15 mm sterile glass petri dishes), then dried at 20.0°C in relative humidity of 50% until visibly dry (20 minutes). For each batch of test substance, using sterile gloves, one dried virus film was wiped with one towelette and held covered at 20.0°C for 30 seconds exposure time. Each carrier was treated in two sections. Each section was treated by wiping the virus film with the towelette over and back 2 times for a total of 4 passes, with minimal overlapping. The area of the towelette used for wiping was rotated to expose a maximal amount of its surface. Following exposure, a 2.00 mL aliquot of test medium was added to each carrier and the dishes were scraped with a cell scraper to re-suspend the contents (10^{-1} dilution). The mixtures were immediately passed through individual Sephadex LH-20 gel columns to detoxify them. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and each dilution assayed for infectivity and/or cytotoxicity. Indicator cells were inoculated in quadruplicate with 100 µL of the dilutions prepared from the test and control groups. Cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and microscopically scored periodically for seven days for the absence or presence of CPE, cytotoxicity and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization.

Note: No protocol amendments or deviations were reported in this study.

15. MRID 511960-19, "Viral Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection." Virus: Bovine Viral Diarrhea virus (surrogate for Human Hepatitis C virus) for product T-Bone (Batch 2290-22 and Batch 2295-009). Study conducted at Accuratus Lab Services by Mary J. Miller, M.T. Study completion date – September 6, 2019. Project Number A27994.

This study was conducted against the NADL strain of Bovine Viral Diarrhea virus (BVDV, ATCC VR-1422) for product T-Bone Formula e0143-166B (Batch 2290-22 and Batch 2295-009). using Accuratus Protocol No. REK01041019.BVD (copy provided). Stock virus obtained from ATCC was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approx. 2000 RPM for 5 minutes at approx. 4°C. The supernatant was removed, aliquoted and the high titer stock virus was stored at ≤-70°C. On the day of use, an aliquot of stock virus (Accuratus Lot NBVD-12) was thawed, maintained at a refrigerated temperature until used in the assay, and contained 5% horse serum as the organic soil load. The culture demonstrated cytopathic effects (CPE) typical of Bovine Viral Diarrhea virus on bovine turbinate (BT) cells. Indicator BT cells originally obtained from ATCC (ATCC CRL-1390) were propagated at Accuratus Lab Services, maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% CO_2. On the day of testing, the cells were observed as having proper cell integrity. Test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Single use towelettes impregnated with the active ingredient were supplied by the Sponsor and used per the Sponsor's direction. The towelettes were folded widthwise two times and lengthwise two times to form an approximate 2 x 2 inch square. The test substance was at exposure temperature prior to use. Films of dried virus were prepared by spreading 200 µL of virus inoculum uniformly over a defined area (approx. 8 x 8 cm) on the bottoms of 6 separate carriers (150 x 15 mm sterile glass petri dishes), then dried at 20.0°C in relative humidity of 40% until visibly dry (20 minutes). For each batch of test substance, using sterile gloves, two dried virus films were wiped with one towelette and held covered at 22.0°C for 30 seconds exposure time. Each carrier was treated in two sections. Each section was treated by wiping the virus film with the towelette over and back 2 times for a total of 4 passes, with minimal overlapping. The area of the towelette used for wiping was rotated to expose a maximal amount of its surface. Following exposure, a 2.00 mL aliquot of test medium was added to each carrier and the dishes were scraped with a cell scraper to re-suspend the contents (10^{-1} dilution). The mixtures were immediately passed through individual Sephadex LH-20 gel columns to detoxify them. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and each dilution assayed for infectivity and/or cytotoxicity. Indicator cells were inoculated in quadruplicate with 100 µL of the dilutions prepared from the input virus control, dried virus control, and test replicates. Cultures were incubated at 37.0°C in a humidified atmosphere of 5-7% CO_2 and were microscopically scored periodically for seven days for the absence or presence of CPE, cytotoxicity and for viability. On the final day of incubation a direct immunofluorescence assay (DFA) was performed using a polyclonal fluorescein conjugated antibody specific for BVDV. (The DFA was performed only on the first inoculated dilution of the test (10^{-1} dilution), and on the 10^{-4} and 10^{-5} dilutions of the dried virus control.) Controls included those for dried virus film recovery, cytotoxicity, neutralization, and negative cell control.

Note: No protocol amendments or deviations were reported for this study.

16. MRID 511960-20, "Viral Efficacy of Pre-Saturated Towelettes for Hard Surface

Disinfection." Virus: Duck Hepatitis B Virus (as a Surrogate Virus for Human Hepatitis B Virus) for product T-Bone (Batch 2290-22 and Batch 2295-009). Study conducted at Accuratus Lab Services by Matt Cantin, B.S. Study completion date – September 9, 2019. Project Number A27997.

This study was conducted against the 9/1/15 strain of the duck Hepatitis B virus (DHBV) for product T-Bone Formula e0143-166B (Batch 2290-22 and Batch 2295-009) using Accuratus Protocol No. REK01041019.DHBV (copy provided). Stock virus containing 100% whole duck serum as organic soil load (from Hepadnavirus Testing Inc.) was stored at ≤-70°C until the day of use. Then two aliquots (Lot 9/1/15 pool) were thawed, combined, maintained at a refrigerated temperature until the assay. The virus was adjusted to contain a 5% fetal bovine serum (FBS) added to the whole duck serum. The stock virus demonstrated fluorescence typical of DHBV on primary duck hepatocytes. Indicator cell cultures: ducklings free of the test virus (screened by Center for AIDS Research at Stanford University) and less than 7 days old were used to obtain purified hepatocytes at Valley Research Institute (VRI). Hepatocytes were obtained by perfusion procedure utilizing S-MEM medium containing collagenase. These were placed in sterile Falcon tubes and transported to Accuratus Lab Services. The hepatocytes were seeded into sterile 12-well labware, maintained and used at appropriate density and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. Test medium used was Leibovitz L-15 medium supplemented with 0.1% glucose, 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin. Single use towelettes impregnated with the active ingredient were supplied by the Sponsor and used per the Sponsor's direction. The towelettes were folded widthwise two times and lengthwise two times to form an approximate 2 x 2 inch square. The test substance was at exposure temperature prior to use. Films of dried virus were prepared by spreading 200 µL of virus inoculum uniformly over a defined area (approx. 8 x 8 cm) on the bottoms of 6 separate carriers (150 x 15 mm sterile glass petri dishes), then dried at 21.0°C in relative humidity of 51.37% until visibly dry (30 minutes). For each batch of test substance, using sterile gloves, two dried virus films were wiped with one towelette and held covered at 21.0°C for 30 seconds exposure time. Each carrier was treated in two sections. Each section was treated by wiping the virus film with the towelette over and back 2 times for a total of 4 passes, with minimal overlapping. The area of the towelette used for wiping was rotated to expose a maximal amount of its surface. Following exposure, a 2.00 mL aliquot of test medium was added to each carrier and the dishes were scraped with a cell scraper to re-suspend the contents (10^{-1} dilution). The mixtures were immediately passed through individual Sephadex LH-20 gel columns to detoxify them. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution (200 µL filtrate + 1.8 mL test medium) and each dilution was assayed for infectivity and/or cytotoxicity. Indicator cells were inoculated in quadruplicate with 250 µL of the dilutions prepared from the input virus control, virus control, and test substances. Prior to inoculation a 1.0 mL aliquot of test medium was added to each well (1.25 mL for negative cell control). Inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2, then a 1.0 mL aliquot of medium was added to each well. Cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 for 9 days, while medium was aspirated from each well and replaced with fresh medium as needed. Then the cultures were scored microscopically for cytotoxicity and then fixed with ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of DHBV. Controls included those for dried virus film recovery, cytotoxicity, neutralization, and negative cell control.

Note: No protocol amendments or deviations were reported in this study.

17. **MRID 511960-21, "Virucidal Hard Surface Efficacy Test For Pre-Saturated or Impregnated Towelettes." Virus: Influenza A Virus (H3N1) for product T-Bone**

(Batch 2315-010 and Batch 2315-011). Study conducted at Microbac Laboratories by Semhar Fanuel. Study completion date – June 18, 2020. Project Number 121-261.

This study was conducted against the A/Shanghai/11/87 (HA) x A/PR/8/34 (NA) subtype of Influenza A Virus (H3N1) for product T-Bone Formula e0143-166B (Batch 2315-010 and Batch 2315-011) using Microbac protocol No. 121.3.01.23.20 (copy provided). Test substance was in ready-to-use pre-saturated towelettes. One carrier per test substance batch was evaluated using a single towelette. Each towelette was folded lengthwise in half two times. The far end was folded up once and this folding was repeated in the same direction so that an additional 4 folds of the same size are made resulting in a total of 5 folds. The outside edges were pulled upward to form a "U" shape when wiping. Handling of the towelette was minimal and no liquid was expressed during folding. Stock virus obtained from BEI Resources was diluted 20-fold in Minimum Essential Medium (MEM), aliquoted, stored at -60 to -90°C, and thawed on the day of the test. Virus was diluted 1.05-fold in Fetal Bovine Serum (FBS) to achieve a 5% organic load. Cell culture dilution medium (DM) was MEM plus 1.0 µg/mL Trypsin. Indicator (host) MDCK cells (ATCC CCL-34, obtained from ATCC) were maintained in cell culture at 36±2°C in 5±3% CO_2 prior to seeding. The host cell plates were prepared 12-30 hours prior to inoculation with test sample. These cells were seeded in 24-well plates at a density of 1.5 x 10^5 cells/mL at 1.0 mL per well. For each batch of test substance, one glass carrier was prepared: a 0.2 mL aliquot of stock virus was spread over an area of approx. 10 in^2 and allowed to dry for 20 minutes at 21°C in 34-37% relative humidity (RH). The carriers were wiped using 2 passes, where 1 pass equaled a back and forth motion for a total of 4 motions. Each carrier was treated in 2 sections to ensure sufficient contact with the entire contaminated area. A new unused area of the folded towelette was exposed for each section, and sections were treated with minimal overlap. After wiping, carriers were exposed for 30 seconds at exposure temperature of 21°C in 33-34% RH. Then the substance was neutralized with 2.0 mL of neutralizer (MEM + 1% FBS + 0.3% Polysorbate 80 + 3% Lecithin + 1% $NaHCO_3$ + 0.025 N NaOH) and the mixture was scraped from the carrier. This was considered the 10^{-1} dilution. Host cells were washed twice with Phosphate Buffered Saline (PBS) prior to the infectivity assay: ten-fold serial dilutions were performed with the neutralized mixture, and selected dilutions were added to host cells at 1.0 mL per well, four wells per dilution, and adsorbed for 21 hours 10 minutes at 36±2°C in 5±3% CO_2. After adsorption, the media was aspirated; the cells were refed with fresh DM and returned to incubation at 36±2°C in 5±3% CO_2 for an additional 5 days. The cultures were then examined microscopically and scored for cytopathic effects (CPE) and test-substance cytotoxic effects. Controls included those for plate recovery, neutralization, cytotoxicity, cell viability and virus stock titer.

Note: No protocol deviations were reported in this study. Protocol amendments reported in this study were reviewed and found to be acceptable.

18. **MRID 511960-22, "Virucidal Hard Surface Efficacy Test For Pre-Saturated or Impregnated Towelettes." Virus: Middle Eastern Respiratory Syndrome Coronavirus (MERS CoV) for product T-Bone (Batch 2315-010 and Batch 2315-011). Study conducted at Microbac Laboratories by Tanya Kapes. Study completion date – May 26, 2020. Project Number 121-262.**

This study was conducted against the EMC/2012 strain of Middle Eastern Respiratory Syndrome Coronavirus (MERS CoV) for product T-Bone Formula e0143-166B (Batch 2315-010 and Batch 2315-011) using Microbac protocol No. 121.4.01.23.20 (copy provided). Test substance was in ready-to-use pre-saturated towelettes. One carrier per test substance batch was evaluated using a single towelette. Each towelette was folded lengthwise in half two times. The far end was folded up once and this folding was repeated in the same direction so

that an additional 4 folds of the same size are made resulting in a total of 5 folds. The outside edges were pulled upward to form a "U" shape when wiping. Handling of the towelette was minimal and no liquid was expressed during folding. Stock virus obtained from BEI Resources was clarified, aliquoted, stored at -60 to -90°C, and thawed on the day of the test. Stock virus contained a 5% organic load. Cell culture dilution medium (DM) was MEM plus 2% FBS. Indicator (host) Vero E6 cells (ATCC CRL-1586, obtained from ATCC) were maintained in cell culture at 36±2°C in 5±3% CO_2 prior to seeding. The host cell plates were prepared 12-30 hours prior to inoculation with test sample. These cells were seeded in 24-well plates at a density of 1.5 x 10^5 cells/mL at 1.0 mL per well. For each batch of test substance, one glass carrier was prepared: a 0.2 mL aliquot of stock virus was spread over an area of approx. 10 in^2 and allowed to dry for 11 minutes at 21°C in 31% relative humidity (RH). The carriers were wiped using 2 passes, where 1 pass equaled a back and forth motion for a total of 4 motions. Each carrier was treated in 2 sections to ensure sufficient contact with the entire contaminated area. A new unused area of the folded towelette was exposed for each section, and sections were treated with minimal overlap. After wiping, carriers were exposed for 30 seconds at exposure temperature of 21°C in 31% RH. Then the substance was neutralized with 2.0 mL of neutralizer (MEM + 10% FBS + 0.3% Polysorbate 80 + 3% HEPES + 1% $NaHCO_3$ + 0.025 N NaOH) and the mixture was scraped from the carrier. This was considered the 10^{-1} dilution. Ten-fold serial dilutions were performed with the neutralized mixture, and selected dilutions were added to host cells at 1.0 mL per well, four wells per dilution, and adsorbed for 24 hours 45 minutes at 36±2°C in 5±3% CO_2. After adsorption, the media was aspirated; the cells were refed with fresh DM and returned to incubation at 36±2°C in 5±3% CO_2 for an additional 8 days. The cultures were then examined and scored for test-substance cytotoxic effects and/or cytopathic effects (CPE). Controls included those for plate recovery, neutralization, cytotoxicity, cell viability and virus stock titer.

Note: No protocol deviations were reported in this study. Protocol amendments reported in this study were reviewed and found to be acceptable.

19. MRID 511960-23, "Virucidal Hard Surface Efficacy Test For Pre-Saturated or Impregnated Towelettes." Virus: Influenza A Virus (H1N2) for product T-Bone (Batch 2315-010 and Batch 2315-011). Study conducted at Microbac Laboratories by Elizabeth Franco. Study completion date – June 16, 2020. Project Number 121-260.

This study was conducted against the A/Swine/Ohio/11SW128/2011 strain of Influenza A Virus (H1N2) for product T-Bone Formula e0143-166B (Batch 2315-010 and Batch 2315-011) using Microbac protocol No. 121.2.01.23.20 (copy provided). Test substance was in ready-to-use pre-saturated towelettes. One carrier per test substance batch was evaluated using a single towelette. Each towelette was folded lengthwise in half two times. The far end was folded up once and this folding was repeated in the same direction so that an additional 4 folds of the same size are made resulting in a total of 5 folds. The outside edges were pulled upward to form a "U" shape when wiping. Handling of the towelette was minimal and no liquid was expressed during folding. Stock virus obtained from BEI Resources was diluted 5-fold in Minimum Essential Medium (MEM), aliquoted, stored at -60 to -90°C, and thawed on the day of the test. Virus was diluted 3.00-fold in MEM and 0.15 mL of Fetal Bovine Serum (FBS) to achieve a 5% organic load. Cell culture dilution medium (DM) was MEM plus 1.0 µg/mL Trypsin. Indicator (host) MDCK cells (ATCC CCL-34, obtained from ATCC) were maintained in cell culture at 36±2°C in 5±3% CO_2 prior to seeding. The host cell plates were prepared 12-30 hours prior to inoculation with test sample. These cells were seeded in 24-well plates at a density of 1.5 x 10^5 cells/mL at 1.0 mL per well. For each batch of test substance, one glass carrier was prepared: a 0.2 mL aliquot of stock virus was spread over an area of approx. 10 in^2 and allowed to dry for 15 minutes at 20°C in 32% relative humidity

(RH). The carriers were wiped using 2 passes, where 1 pass equaled a back and forth motion for a total of 4 motions. Each carrier was treated in 2 sections to ensure sufficient contact with the entire contaminated area. A new unused area of the folded towelette was exposed for each section, and sections were treated with minimal overlap. After wiping, carriers were exposed for 30 seconds at exposure temperature of 20°C in 32% RH. Then the substance was neutralized with 2.0 mL of neutralizer (MEM + 1% FBS + 0.3% Polysorbate 80 + 3% HEPES + 1% $NaHCO_3$ + 0.025 N NaOH) and the mixture was scraped from the carrier. This was considered the 10^{-1} dilution. Host cells were washed twice with Phosphate Buffered Saline (PBS) prior to the infectivity assay: Ten-fold serial dilutions were performed with the neutralized mixture, and selected dilutions were added to host cells at 1.0 mL per well, four wells per dilution, and adsorbed for 22 hours 25 minutes at 36±2°C in 5±3% CO_2. After adsorption, the media was aspirated; the cells were refed with fresh DM and returned to incubation at 36±2°C in 5±3% CO_2 for an additional 5 days. The cultures were then examined and scored for test-substance cytotoxic effects and/or cytopathic effects (CPE). Controls included those for plate recovery, neutralization, cytotoxicity, cell viability and virus stock titer.

Note: No protocol deviations were reported in this study. Protocol amendments reported in this study were reviewed and found to be acceptable.

20. MRID 511960-24, "Viral Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection." Virus: Herpes simplex virus type 2 for product T-Bone (Batch 2315-10 and Batch 2315-011). Study conducted at Analytical Lab-Group-Midwest by Mary J. Miller, M.T. Study completion date – April 8, 2020. Project Number A29144.

This study was conducted against the G strain of Herpes simplex virus type 2 (ATCC VR-734) for product T-Bone Formula #e0143-166B (Batch 2315-10 and Batch 2315-011) using Accuratus Protocol No. REK01011720.HSV2 (copy provided). Stock virus obtained from ATCC was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approx. 2000 RPM for 5 minutes at approx. 4°C. The supernatant was removed, aliquoted and the high titer stock virus was stored at ≤-70°C. On the day of use, an aliquot of stock virus (Accuratus Lot H2-79) was thawed, maintained at a refrigerated temperature until used in the assay, and contained 5% fetal bovine serum (FBS) as the organic soil load. The culture demonstrated cytopathic effects (CPE) typical of Herpes simplex virus on Vero cells. Indicator Vero cells originally obtained from ATCC (ATCC CCL-81) were propagated at Accuratus Lab Services, seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. On the day of testing, the cells were observed as having proper cell integrity and confluency. Test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat inactivated FBS, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Single use towelettes impregnated with the active ingredient were supplied by the Sponsor and used per the Sponsor's direction. The towelettes were folded widthwise two times and lengthwise two times to form an approximate 2 x 2 inch square. These were pre-equilibrated to exposure temperature prior to use. Films of dried virus were prepared by spreading 200 µL of virus inoculum uniformly over a defined area (approx. 8 x 8 cm) on the bottoms of 3 separate carriers (150 x 15 mm sterile glass petri dishes), then dried at 20.0°C in relative humidity of 50% until visibly dry (20 minutes). For each batch of test substance, using sterile gloves, one dried virus film was wiped with one towelette and held covered at 21.0°C for 30 seconds exposure time in 15.20% RH. Each carrier was treated in two sections. Each section was treated by wiping the virus film with the towelette over and back 2 times for a total of 4 passes, with minimal overlapping. The area of the towelette used for wiping was rotated to expose a maximal amount of its surface. Following exposure, a 2.00 mL aliquot of test medium was added to each carrier and the dishes were scraped with a cell

scraper to re-suspend the contents (10^{-1} dilution). The mixtures were immediately passed through individual Sephadex LH-20 gel columns to detoxify them. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and each dilution assayed for infectivity and/or cytotoxicity. Indicator cells were inoculated in quadruplicate with 100 µL of the dilutions prepared from the test and control groups. Cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and microscopically scored periodically for seven days for the absence or presence of CPE, cytotoxicity and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization.

Note: No protocol amendments or deviations were reported in this study.

21. MRID 511960-25, "Virucidal Hard Surface Efficacy Test For Pre-Saturated or Impregnated Towelettes." Virus: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) (COVID-19 Virus) for product T-Bone (Batch 2315-010 and Batch 2315-011 and Batch 2315-21). Study conducted at Microbac Laboratories by Tanya Kapes. Study completion date – June 25, 2020. Project Number 121-294.

This study was conducted against the USA-WA1/2020 strain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) (COVID-19 Virus) for product T-Bone Formula e0143-166B (Batch 2315-010 and Batch 2315-011 and Batch 2315-21) using Microbac protocol No. 121.23.03.23.20 (copy provided). Test substance was in ready-to-use pre-saturated towelettes. One carrier per test substance batch was evaluated using a single towelette. Each towelette was folded widthwise two times and lengthwise two times to form an approximate 2 x 2 inch square. Handling of the towelette was minimal and no liquid was expressed during folding. Stock virus obtained from BEI Resources was clarified, aliquoted, stored at -60 to -90°C, and thawed on the day of the test. Stock virus contained a 5% organic load. Cell culture dilution medium (DM) was MEM plus 2% Newborn Calf Serum (NCS). Indicator (host) Vero E6 cells (ATCC CRL-1586, obtained from ATCC) were maintained in cell culture at 36±2°C in 5±3% CO_2 prior to seeding. The host cell plates were prepared 12-30 hours prior to inoculation with test sample. These cells were seeded in 24-well plates at a density of 1.5 x 10^5 cells/mL at 1.0 mL per well. For each batch of test substance, one glass carrier was prepared: a 0.2 mL aliquot of stock virus was spread over an area of approx. 10 in^2 and allowed to dry for 20 minutes at 21°C in 26% relative humidity (RH). The carriers were wiped using 3 passes, where 1 pass equaled a back and forth motion for a total of 6 motions. Each carrier was treated in 2 sections to ensure sufficient contact with the entire contaminated area. A new unused area of the folded towelette was exposed for each section, and sections were treated with minimal overlap. After wiping, carriers were exposed for 30 seconds at exposure temperature of 21°C in 26% RH. Then the substance was neutralized with 2.0 mL of neutralizer (MEM + 7% NCS + 2% HEPES + 1% $NaHCO_3$ + 0.5% Polysorbate 80 + 0.025 N NaOH) and the mixture was scraped from the carrier. This was considered the 10^{-1} dilution. Ten-fold serial dilutions were performed with the neutralized mixture, and selected dilutions were added to host cells at 1.0 mL per well, four wells per dilution, and adsorbed for 23 hours 49 minutes at 36±2°C in 5±3% CO_2. After adsorption, the media was aspirated; the cells were refed with fresh DM and returned to incubation at 36±2°C in 5±3% CO_2 for an additional 8 days. The cultures were then examined and scored for test-substance cytotoxic effects and/or cytopathic effects (CPE). Controls included those for plate recovery, neutralization, cytotoxicity, cell viability and virus stock titer.

Note: No protocol deviations were reported in this study. Protocol amendments reported in this study were reviewed and found to be acceptable.

22. MRID 511960-26, "Fungicidal Pre-Saturated Towelettes for Hard Surface Disinfection." Test organism: *Candida albicans* (ATCC 10231) for product T-Bone (Batch 2315-010 and Batch 2315-011). Study conducted at Analytical Lab-Group-Midwest by James Walrath, B.S. Study completion date – April 13, 2020. Project Number A29142.

This study was conducted against *Candida albicans* (ATCC 10231) for product T-Bone Formula e0143-166B (Batch 2315-010 and Batch 2315-011) using Accuratus Laboratory Protocol No. REK01011720.FTOW.1 (copy provided). From stock, sufficient Sabouraud Dextrose agar plates were inoculated and incubated for 2 days at 25-30°C (29.0°C). Following incubation, the organism was suspended in Butterfield's Buffer using a sterile device to target a spectrophotometer absorbance reading of 2.00 (1.926) at 620 nm. The test culture was thoroughly mixed prior to use. A 0.10 mL aliquot of Fetal Bovine Serum (FBS) was added to 1.90 mL of prepared culture to yield a 5% organic soil load. Individual glass slide carriers (in Petri dishes matted with two pieces of filter paper) were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide. The dish was covered immediately, and the procedure repeated until all slides were individually inoculated. The culture was vortex mixed periodically during inoculation as necessary. The carriers were allowed to dry for 30 minutes at 25-30°C in 63% relative humidity and appeared visibly dry. Carriers were used in the test procedure within 2 hours of drying. Test substance was in ready-to-use pre-saturated towelettes. Each towelette was folded in half lengthwise twice and rolled up 5 times prior to use. For each batch of test substance, one towelette was used to wipe the contaminated portions of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during wiping. Each inoculated carrier was treated by passing the towelette over the carrier surface back and forth for a total of 4 passes. Following exposure, each carrier was held for 4 minutes exposure at room temperature (20°C) and 21% relative humidity. At the end of the exposure time, the excess liquid was drained off the carrier without touching the carrier to the Petri dish or filter paper. The wiping procedure was performed at staggered intervals to allow for the prescribed exposure time, and the procedure was performed within ±5 seconds of the exposure time following a calibrated timer. Each carrier was then drained of excess liquid and then transferred using sterile forceps and following staggered intervals to 20 mL aliquots of neutralizing subculture medium (Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80), and the vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours (47 hours) at 25-30°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, carrier population and neutralization confirmation.

Note: Protocol amendments and deviations reported in this study were reviewed and found to be acceptable.

23. MRID 511960-27, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)". Test Organism: *Campylobacter jejuni* (ATCC 33560) for product T-Bone US at LCL (777-139), Lots # 2290-022 and # 2295-009. Study conducted at Accuratus Lab Services by Kristin Hunt, B.S. Study completion date – September 10, 2019. Project Number A28008.

This study was conducted against *Campylobacter jejuni* (ATCC 33560). Two lots (Lot Nos. 2290-022 and 2295-009) of the product, T-Bone US at LCL e0143-166B (777-139), were tested using Accuratus Lab Protocol No. REK01041019.NFS.2. The product was received ready-to-use as a pre-saturated towelette. From frozen stock, a stock culture plate of test

organism was created on BAP and incubated for 2-5 days (2 days) at 35-37°C (36.0°C) under microaerophilic conditions (CampyPak™ Plus). From the stock culture BAP plate, multiple BAP plates were inoculated and incubated for 2-3 days (2 days) at 35-37°C (36.0°C) under microaerophilic conditions (CampyPak™ Plus). Following incubation, a bacterial culture suspension was prepared by swabbing bacterial growth and placing the swab in Fluid Thioglycollate Medium to yield ~1.0 x 10^8 CFU/mL. The culture was adjusted using Fluid Thioglycollate Medium to reach a spectrophotometer absorbance reading of 1.352 at 620 nm. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of prepared culture to achieve a 5% organic soil load. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor spreading the inoculum to within ~ 3 mm of the edge of the carrier. The inoculated carriers were dried for 14 minutes at 25-30°C (26.2-26.9°C) and 65% relative humidity with the Petri dish lids slightly ajar. A constant humidity chamber was used to ensure uniform humidification conditions and overcome slow re-equilibration. After drying, each of the five test carriers were wiped with the saturated towelette at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (21°C) and 53% relative humidity for 10 seconds in a horizontal and undisturbed fashion. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 0.28% Lecithin + 2.0% Tween 80) using identical staggered intervals. The jars were vortex-mixed for 10-15 seconds to suspend the surviving organisms. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution (10^0) were plated onto the recovery agar plate medium (Tryptic Soy Agar with 5% Sheep Blood, BAP). The plates were incubated at 35-37°C (36.0°C) for 2-7 days (4 days) under microaerophilic conditions. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, neutralization confirmation, carrier population, and inoculum count.

Note: No protocol deviations were required; protocol amendments in this study were reviewed and found to be acceptable. Testing was performed on July 5, 2019 resulted in carrier population control failure. Testing was repeated on July 29, 2020 resulted in valid test results.

24. MRID 511960-28, "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Pre-Saturated Towelette Product Application)". Test Organisms: *Klebsiella aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538) for product T-Bone US (777-139), Lots # 2315-010, #2315-011 and # 2315-021. Study conducted at Analytical Lab Group-Midwest by Jamie Herzan, B.S. Study completion date – April 15, 2020. Project Number A29145.

This study was conducted against *Klebsiella aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 2315-010, 2315-011 and 2315-021) of the product, T-Bone US e0143-166B (777-139), were tested using Analytical Lab Group-Midwest Protocol No. REK01011720.NFS. The product was received ready-to-use as a pre-saturated towelette. From a stock slant, no more than 5 transfers from original stock and ≤1 month old, 10 mL of culture broth was inoculated. From this initial broth suspension, a minimum of 3 daily transfers using 1 loopful (10 µL) of culture into 10 mL of culture media was performed on consecutive days prior to use. The *Klebsiella aerogenes* daily transfer was incubated at 25-32°C (29.0°C) for 24±2 hours using Tryptic Soy Broth growth medium. The *Staphylococcus aureus* daily transfer was incubated at 35-37°C (36.0°C) for 24±2 hours using Nutrient Broth growth medium. A 48-54 hour (48 hour) culture that was incubated at 25-32°C (29.0°C) and a 48-54 hour (48 hour) culture of *Staphylococcus aureus* that was incubated at 35-37°C (36.0°C) were vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds

of each culture was removed and transferred to individual sterile vessels for use in testing. The *Staphylococcus aureus* culture was centrifuge concentrated at 3500 RPM for 10 minutes. A total of 25.0 mL of culture was concentrated to 5.0 mL. The cultures were mixed thoroughly prior to use. A 0.10 mL aliquot of fetal bovine serum was added to 1.90 mL of each prepared culture to achieve a 5% organic soil load. Sterile carriers (~1 inch x 1 inch plain 100% cotton weave fabric) were inoculated with 0.03 mL (30.0 µL) of culture using a calibrated pipettor spreading the inoculum evenly about the carrier. The inoculated carriers were dried for 30 minutes at 35-37°C (36.0-36.2°C) and 41-42% relative humidity with the Petri dish lids intact. A constant humidity chamber was used to ensure uniform humidification conditions and overcome slow re-equilibration. After drying, each of the five test carriers were wiped with the saturated towelette at staggered intervals. The area of the towelette used was rotated and each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth two times for a total of four passes. The carriers were allowed to expose at room temperature (20°C) and 19% relative humidity for 3 minutes 50 seconds. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 0.28% Lecithin + 2.0% Tween 80) using identical staggered intervals. The carriers were vortex-mixed for 10-15 seconds to ensure complete elution of the test organism. Glass beads were utilized to aid in organism recovery from the modified fabric substrate. Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution (10^0) were plated onto the recovery agar plate medium (Tryptic Soy Agar with 5% Sheep Blood, BAP). The *Staphylococcus aureus* plates were incubated at 35-37°C (36.0°C) for 48±4 hours (44.5 hours). The *Klebsiella aerogenes* plates were incubated at 25-32°C (29.0°C) for 48±4 hours (44.5 hours). Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, neutralization confirmation, carrier population, and inoculum count.

Note: No protocol amendments or deviations were reported in this study. Testing was performed on February 3, 2020 resulted in carrier population control failures for both test organisms. Testing was repeated on February 12, 2020, using different test organism adjustments which resulted in valid test results.

IV. RESULTS

Disinfection – Bactericidal

MRID Number	Organism	Batch No.	No. of Carriers Exhibiting Growth/ Total No. Tested	Carrier Population CFU/carrier (Average Log_{10})
3 Minute 50 second Exposure Time (5% Soil Load; Ready-to-use Pre-saturated towelette)				
511960-05	*Bordetella pertussis* (ATCC 12743)	2290-022	0/10	4.72
		2290-023	0/10	
511960-06	*Vibrio cholerae* (ATCC 14035)	2290-022	0/10	4.72
		2290-023	0/10	
511960-07	*Streptococcus mutans* (ATCC 25175)	2290-022	0/10	5.52
		2290-023	0/10	
511960-08	*Shigella dysenteriae* (ATCC 11835)	2290-022	0/10	5.14
		2290-023	0/10	
511960-09	*Enterococcus faecium* Vancomycin Resistant (ATCC BAA-2320)	2315-010	0/10	5.10
		2315-011	0/10	4.88
511960-10	*Escherichia coli* 3rd Generation Cephalosporin Resistant (ATCC BAA-2780)	2315-010	0/10	5.01
		2315-011	0/10	
511960-11	*Klebsiella pneumoniae* 3rd Generation Cephalosporin Resistant (ATCC BAA-2784)	2315-010	0/10	5.32
		2315-011	0/10	4.72
511960-12	*Staphylococcus aureus* Vancomycin Intermediate Resistant VISA (CDC HIP 5836)	2315-010	0/10	4.00
		2315-021	0/10	
511960-13	*Escherichia coli* Carbapenem Resistant (CDC 81371)	2315-010	0/10	4.57
		2315-011	0/10	
511960-14	*Staphylococcus aureus* Vancomycin Resistant VRSA (NARSA VRS1)	2315-010	0/10	4.15
		2315-011	0/10	
511960-15	*Klebsiella aerogenes* Multidrug Resistant (AR-Bank 0547)	2315-010	0/10	4.60
		2315-011	0/10	

MRID Number	Organism	Batch No.	No. of Carriers Exhibiting Growth/ Total No. Tested	Carrier Population CFU/carrier (Average Log_{10})
3 Minute 50 second Exposure Time (5% Soil Load; Ready-to-use Pre-saturated towelette)				
511960-16	*Acinetobacter baumannii* Carbapenem Resistant (ATCC BAA-2901)	2315-010	0/10	4.69
		2315-011	0/10	5.13
511960-17	*Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705)	2315-010	0/10	4.69
		2315-011	0/10	

Disinfection – Virucidal

30 Second Exposure Time (5% Soil Load (*except where noted) Ready-to-use Pre-saturated towelette)						
MRID	**Organism**	**Results**				
		Complete Inactivation Dilutions	**Lot # or Batch #**	**Log_{10} $TCID_{50}$/carrier**	**Dried Virus Control (Log_{10}/$TCID_{50}$ carrier)**	**Log_{10} Reduction**
511960-18	Herpes simplex virus type 1 (ATCC VR-733)	10^{-1} to 10^{-7}	2290-022	$\leq 10^{0.80}$	5.55	≥4.75
			2295-009	$\leq 10^{0.80}$		
511960-19	Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C) (ATCC VR-1422)	10^{-1} to 10^{-4}	2290-022	$\leq 10^{0.80}$	5.37 **	≥4.57
			2295-009	$\leq 10^{0.80}$		
511960-20	Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus), Strain 9/1/15 *	10^{-1} to 10^{-4}	2290-022	$\leq 10^{0.40}$	5.29 **	≥4.89
			2295-009	$\leq 10^{0.40}$		
511960-21	Influenza A virus (H3N1), Strain: A/Shanghai/11/87 (HA) x A/PR/8/34 (NA)	10^{-4} to 10^{-7}	2315-010	$\leq 10^{2.80}$	6.05	≥3.25
			2315-011	$\leq 10^{2.80}$		
511960-22	Middle Eastern Respiratory Syndrome Coronavirus (MERS CoV), Strain EMC/2012	10^{-3} to 10^{-7}	2315-010	$\leq 10^{1.80}$	5.05	≥3.25
			2315-011	$\leq 10^{1.80}$		
511960-23	Influenza A virus (H1N2), Strain: A/Swine/Ohio/ 11SW128/2011	10^{-4} to 10^{-7}	2315-010	$\leq 10^{2.80}$	5.80	≥3.00
			2315-011	$\leq 10^{2.80}$		
511960-24	Herpes simplex virus type 2 (ATCC VR-734) Strain G	10^{-1} to 10^{-7}	2315-010	$\leq 10^{0.80}$	4.80	≥4.00
			2315-011	$\leq 10^{0.80}$		
511960-25	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020	10^{-3} to 10^{-7}	2315-010	$\leq 10^{1.80}$	4.80	≥3.00
			2315-011	$\leq 10^{1.80}$		
			2315-021	$\leq 10^{1.80}$		

* Soil load was 100% Whole Duck Serum

** Average of replicates

Disinfection – Fungicidal

4 Minutes Exposure Time (5% Soil Load; Ready-to-use Pre-saturated towelette)				
MRID Number	**Organism**	**Carrier Population Control (Average Log_{10}/Carrier)**	**Lot # or Batch #**	**Results (Carriers Showing Growth)**
511960-26	*Candida Albicans* (ATCC 10231)	5.29	2315-010	0/10
			2315-011	0/10

Non-Food Contact Sanitizer (511960-27) and Soft Surface Sanitizer (511960-28)

MRID Number	Organism	Batch	CFU / Carrier Geometric Mean Average Log_{10}	Percent Reduction	Carrier Population Average CFU / Carrier (Log_{10})
10 Second Exposure Time (5% Soil Load; Ready-to-use Pre-saturated towelette)					
511960-27	*Campylobacter jejuni* (ATCC 33560)	2290-022	$<2.00 \times 10^2$ <2.30	>99.99%	1.02×10^7 (7.01)
		2295-009	$<2.00 \times 10^2$ <2.30	>99.99%	
3 Minute 50 Second Exposure Time (5% Soil Load; Ready-to-use Pre-saturated towelette)					
511960-28	*Klebsiella aerogenes* (ATCC 13048)	2315-010	$<2.00 \times 10^1$ <1.30	>99.999%	3.89×10^6 (6.59)
		2315-011			
		2315-021			
	Staphylococcus aureus (ATCC 6538)	2315-010	$<2.00 \times 10^1$ <1.30	>99.999%	4.17×10^6 (6.62)
		2315-011			
		2315-021			

V. CONCLUSIONS

1. The submitted efficacy data **supports** the label claims for the ready to use test substance (tested at the LCL) as a disinfectant against the following bacteria on hard, non-porous surfaces with a 5% soil load for a 3-minute 50-second contact time at room temperature (19-25.7°C):

Bordetella pertussis (ATCC 12743)	511960-05
Vibrio cholerae (ATCC 14035)	511960-06
Streptococcus mutans (ATCC 25175)	511960-07
Shigella dysenteriae (ATCC 11835)	511960-08
Vancomycin-Resistant *Enterococcus faecium* (ATCC BAA-2320)	511960-09
3rd Generation Cephalosporin-Resistant *Escherichia coli* (ATCC BAA-2780)	511960-10
3rd Generation Cephalosporin-Resistant *Klebsiella pneumoniae* (ATCC BAA-2784)	511960-11
Vancomycin Intermediate Resistant *Staphylococcus aureus* VISA (CDC HIP 5836)	511960-12
Carbapenem Resistant *Escherichia coli* (CDC 81371)	511960-13
Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1)	511960-14
Multidrug-Resistant *Klebsiella aerogenes* (AR-Bank 0547)	511960-15
Carbapenem-Resistant *Acinetobacter baumannii* (ATCC BAA-2901)	511960-16
Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)	511960-17

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data **supports** the label claims for the ready to use test substance (tested at the LCL) as a disinfectant against the following viruses on hard, non-porous surfaces with 5% soil load (soil load was 5% in 100% duck serum for duck hepatitis B virus) for a 30 second contact time at room temperature (20-22°C):

F(1) strain of Herpes simplex virus type 1 (ATCC VR-733)	511960-18
NADL strain of Bovine Viral Diarrhea virus (BVDV, ATCC VR-1422)	511960-19
Duck Hepatitis B virus (DHBV, ATCC-1422)	511960-20
A/Shanghai/11/87 (HA) x A/PR/8/34 (NA) subtype of Influenza A Virus (H3N1)	511960-21
EMC/2012 strain of Middle Eastern Respiratory Syndrome Coronavirus (MERS CoV)	511960-22
A/Swine/Ohio/11SW128/2011 strain of Influenza A Virus (H1N2)	511960-23
G strain of Herpes simplex virus type 2 (ATCC VR-734)	511960-24
USA-WA1/2020 strain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) (COVID-19 Virus)	511960-25

Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

3. The submitted efficacy data **supports** the label claims for the ready to use product (tested at the LCL) as a disinfectant against the following fungus on hard, non-porous non-food contact surfaces with a 5% soil load for a 4-minute contact time at room temperature (20°C):

   Candida albicans (ATCC 10231) 511960-26

   Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

4. The submitted efficacy data **supports** the label claims for the ready to use product (tested at the LCL) as a sanitizer against the following bacteria on hard, non-porous non-food surfaces with a 5% soil load for a 10 second contact time at room temperature (21°C):

   Campylobacter jejuni (ATCC 33560) 511960-27

   Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

5. The submitted efficacy data **supports** the label claims for the ready to use product (tested at the LCL) as a soft surface sanitizer against the following bacteria on 100% cotton weave fabric with a 5% soil load for a 3 minute 50 second contact time at room temperature (20°C):

   Klebsiella aerogenes (ATCC 13048) 511960-28
   Staphylococcus aureus (ATCC 6538) 511960-28

   Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

VI. LABEL RECOMMENDATIONS (Label date: July 1, 2020)

1. The label claims that the ready-to-use product is a one-step disinfectant against the following bacteria on hard non-porous surfaces with a contact time of 4 minutes:

Bordetella pertussis (ATCC 12743)	511960-05
Vibrio cholerae (ATCC 14035)	511960-06
Streptococcus mutans (ATCC 25175)	511960-07
Shigella dysenteriae (ATCC 11835)	511960-08
Vancomycin-Resistant *Enterococcus faecium* (ATCC BAA-2320)	511960-09
3rd Generation Cephalosporin-Resistant *Escherichia coli* (ATCC BAA-2780)	511960-10
3rd Generation Cephalosporin-Resistant *Klebsiella pneumoniae* (ATCC BAA-2784)	511960-11
Vancomycin Intermediate Resistant *Staphylococcus aureus* VISA (CDC HIP 5836)	511960-12
Carbapenem Resistant *Escherichia coli* (CDC 81371)	511960-13
Vancomycin Resistant *Staphylococcus aureus* - VRSA (NARSA VRS1)	511960-14
Multidrug-Resistant *Klebsiella aerogenes* (AR-Bank 0547)	511960-15
Carbapenem-Resistant *Acinetobacter baumannii* (ATCC BAA-2901)	511960-16
Carbapenem Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)	511960-17

These claims are acceptable as they are supported by the submitted data.

2. The label claims that the ready-to-use product is a one-step disinfectant against the following viruses on hard non-porous surfaces with a contact time of 30 seconds:

F(1) strain of Herpes simplex virus type 1 (ATCC VR-733)	511960-18
NADL strain of Bovine Viral Diarrhea virus (BVDV, ATCC VR-1422)	511960-19
Duck Hepatitis B virus (DHBV, ATCC-1422)	511960-20
A/Shanghai/11/87 (HA) x A/PR/8/34 (NA) subtype of Influenza A Virus (H3N1)	511960-21
EMC/2012 strain of Middle Eastern Respiratory Syndrome Coronavirus (MERS CoV)	511960-22
A/Swine/Ohio/11SW128/2011 strain of Influenza A Virus (H1N2)	511960-23
G strain of Herpes simplex virus type 2 (ATCC VR-734)	511960-24
USA-WA1/2020 strain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) (COVID-19 Virus)	511960-25

These claims are acceptable as they are supported by the submitted data.

3. The label claims that the ready-to-use product is a one-step disinfectant against the following fungus on hard non-porous surfaces with a contact time of 4 minutes:

Candida albicans (ATCC 10231)	511960-26

This claim is acceptable as it is supported by the submitted data.

4. The label claims that the ready-to-use product is a sanitizer against the following bacterium on hard non-porous surfaces with a contact time of 10 seconds:

Campylobacter jejuni (ATCC 33560)	511960-27

This claim is acceptable as it is supported by the submitted data.

5. The label claims that the ready-to-use product is a sanitizer against the following bacteria on soft surfaces with a contact time of 4 minutes:

Klebsiella aerogenes (ATCC 13048)	511960-28
Staphylococcus aureus (ATCC 6538)	511960-28

These claims are acceptable as they are supported by the submitted data.

6. Make the following changes to the proposed label:
    a. Throughout the label,
        i. Refrain from excessive bracketing, and when bracketing is necessary ensure the full claim is appropriate in all iterations.
        ii. Qualify all one step disinfection and/or sanitization claims (e.g. “one step disinfection” or “cleans as it disinfects” or similar) with “when used according to disinfection instructions or sanitization instructions” as appropriate
        iii. Qualify all instances of “soft surfaces” or “clothing” or similar claims with “100% cotton”, as supported by testing. The data does not support other soft surface types.
        iv. Qualify all one step claims such as “Deodorizes (and sanitizes***) as it cleans” with “when used according to sanitization directions” or disinfection directions as appropriate
        v. Remove “2019 Novel coronavirus” as the virus has been officially renamed to SARS CoV 2. “Novel” is a temporary descriptor, not a consistent identifier for this virus.
        vi. Qualify “all around the house” claims with when used according to appropriate directions for use (Page 12 and 13)
        vii. Qualify the below disinfection claims with, “on hard, non-porous surfaces”
            1. (Multi action)(Multi purpose) disinfectant and cleaner (page 11)
            2. Disinfects and easily cleans (your) (house) (home) (page 12)
            3. Disinfection and (effortless) (with one hand) cleaning (Page 12)
            4. Hospital Type (Disinfectant) (Disinfection) (Page 13)
    b. Under the “Marketing Claims” section(s)
        i. Either remove “Easy” from all Easy claims or specify for cleaning only, as easy when referring to disinfection or sanitization can imply heighted efficacy.
    c. On page 11, specify surface types in the claim, “One wipe, many surfaces”
    d. On Page 12, Remove parenthesis from “(hard non-porous surfaces)” in “(Effectively) works on (many) (most) (a wide range of) (household) (kitchen) (bathroom) (hard non-porous surfaces)”
    e. On Page 16, edit “Staph” to include the entire binomial name of the organism or qualify it with the appropriate symbol similar to how it is throughout the majority of the label
    f. On Page 18
        i. qualify all SARS-CoV-2 claims with when used according to disinfection instructions
    g. On page 19,
        i. remove “semi critical medical device or medical equipment surfaces” as this use falls under FDA jurisdiction.
        ii. for each set of use directions, remove parenthesis around “Surface must remain wet for the entire contact time” or otherwise revise instructions to indicate surfaces should be visibly wet for the duration of the contact time.
    h. On page 31, to ensure EVP claims are clear and easy to understand for the end-user, please select the minimum number of viruses to support each EVP category.

Note to PM: The efficacy review dated March 19, 2020 for this product did not assess emerging viral pathogen claims. Please verify that the registrant has provided a Terms of Registration to support EVP claims.

i. Note to registrant: Data should be submitted to substantiate base fungi prior to adding additional fungi.